This Month in Genetics

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Out of the Ordinary

Genetic testing decisions are often based on a comparison of a patient to the classic presentation of a mutation. Does the patient have the appropriate family history? How well do they match the list of expected phenotypes? Which genes are most likely to be mutated? We have already seen with comparative genome hybridization arrays that the use of less-biased approaches to genetic diagnosis can broaden the phenotype associated with certain genetic disorders and increase the diagnostic yield in certain classes of patients. Even though it is in its infancy, clinical use of whole genome sequencing is likely to have a similar effect, as illustrated in two recent *JAMA* papers. In both cases, known genetic defects were uncovered, so it is not that the relevant mutations could not have been found via conventional techniques. However, wading through the candidates gene-by-gene and using conventional guidelines would probably have meant these atypical situations would have gone unidentified. In one paper, by Link et al., a woman with early onset breast and ovarian cancer and with no family history of cancer was found to have a presumably de novo mutation in *TP53*. Although she had been tested for mutations in *BRCA1* and *2*, she did not meet the criteria for Li-Fraumeni syndrome and had not been offered *TP53* mutation analysis. The diagnosis of Li-Fraumeni syndrome in this woman has serious implications for cancer risks to her children. In another, by Welch et al., whole-genome sequencing was completed on a leukemia patient within 7 weeks and resulted in a drastic change in patient management. The woman presented with what appeared to be acute promyelocytic leukemia (APL), which is often successfully treated with chemotherapy plus all-trans retinoic acid when the disease is due to chromosome translocations that lead to gene fusions involving *RARA*. Standard cytogenetic analysis indicated a poor prognosis; although the leukemic cells had a complicated series of chromosome rearrangements, they lacked the standard *RARA* fusion. She was thus referred for allogeneic stem cell transplantation. Before this was performed, whole-genome sequencing of her tumor sample identified an unusual insertion event in *RARA* that led to a classic APL-causing fusion gene. As a result, the patient received all-trans retinoic acid instead of a stem cell transplant and has a much better prognosis than originally believed. Although at ~$20,000 per genome whole-genome sequencing is still far from routine in the clinic, it is now technically feasible in a time-frame that is appropriate for clinical decision-making.

Link et al. JAMA 305: 1568–1576.

Welch et al. JAMA 305: 1577–1584.

Nature and Nurture in Lung Disease

Although there are familial forms of pulmonary fibrosis, this disorder of lung scarring is a complex trait, and the known genetic underpinnings account for a minority of cases. Seibold et al. recently used linkage analysis followed by association studies to uncover a new type of protein that contributes to pulmonary fibrosis, a component of the bronchial mucous. The genetic variant associated with pulmonary fibrosis is upstream of *MUC5B* and is associated with increased expression of the gene in control individuals. The fact that this increased mucin expression might contribute to disease pathogenesis is supported by the finding that *MUC5B* is overexpressed in individuals with idiopathic pulmonary fibrosis regardless of their genotype at this SNP, and dense accumulations of MUC5B protein can be seen in the bronchi of these individuals. Although genetic and environmental factors have to come together to yield lung disease, some affected people might have a genetic predisposition to overproduction of this mucin, whereas in others the environmental contribution might yield the same result.

Seibold et al. NEJM 364: 1503–1512.

This Story Gets Cilia and Cilia

The number of genes and disorders that belong to the ciliopathy class seems ever-increasing and serves as a model for studying gene-gene interactions in the contribution to disease. A recent paper adds more data to the ciliopathy story. Although the fetal hydrolethalus and acrocallosal syndromes were suspected to be ciliopathies based on phenotype, which in both disorders includes polydactyly, abnormalities of the brain, and cleft palate, it was not until the work by Putoux et al. that this connection was cemented. SNP arrays in a consanguineous family affected by fetal hydrolethalus identified a region of homozygosity on the long arm of chromosome 15 in affected fetuses. The relevant gene, *KIF7*, is a cilia protein that was found to be mutated in this family as well as in a series of families affected by acrocallosal syndrome. In tissue from affected individuals, many direct and secondary targets of GLI transcription factors are upregulated, a finding that fits with the

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role of KIF7 in Hedgehog signaling. Although homozygous mutations in *KIF7* cause these rare syndromes, additional data implicate heterozygous *KIF7* variation in the development of other ciliopathies. In a sample of 130 individuals with ciliopathies caused by mutations in other genes, hypomorphic missense changes in *KIF7* were significantly enriched compared to controls, suggesting that *KIF7* variation exacerbates the phenotype caused by other ciliary defects.

Putoux et al. Nature Genetics. Published online May 8, 2011. 10.1038/ng.826.

Fostering Tolerance

I imagine a lot of us were taught that the sickle cell trait protects against malaria because it makes red blood cells fragile, thereby inhibiting replication of the parasite. Ferreira et al. recently proposed a completely new model, based on experiments in mice expressing human hemoglobin, to explain the protective effect of the sickle cell allele. It turns out, at least in this mouse model of severe malaria, that the protection is not a result of reduced parasite load. Instead, the HbS allele fosters immune tolerance, thereby lessening the risk of cerebral malaria, an immunologically-mediated, severe complication of the infection. More than a single factor contributes to this tolerance. For one, a low, but elevated, level of free heme from HbS induces expression of the heme oxygenase-1 (HO-1) via the Nrf2 transcription factor. HO-1 catabolizes the free heme to protect against its cytotoxicity, thereby preventing tissue damage. This reaction also produces carbon monoxide, which in turn prevents further release of free heme as well as having anti-inflammatory properties. Second, in the brain, HO-1 inhibits expression of chemokines that contribute to the pathogenesis of cerebral malaria. Finally, through a mechanism that does not involve HO-1, the HbS allele prevents expansion of CD8+T cells that promote development of cerebral malaria. Thus, it appears that the sickle cell trait might not increase malaria survival by reining in the parasite as much as it reins in the host response to reduce the severe complications of the infection.

Ferreira et al. Cell 145: 398–409.

Hit Me One Time. Hit Me Two Times

How do you sort through the reams of sequence data that you get from a whole-genome or whole-exome sequence project? O'Roak et al. decided to focus on de novo mutation events through use of parent-child trios in the hopes of explaining sporadic cases of autism. In 20 such trios, they found 11 de novo events that were predicted to alter the encoded protein, a manageable number to wade through. Of these, four are identified as potentially causative. These mutations were found in *GRIN2B*, *FOXP1*, *SCN1A*, and *LAMC3*. Seems pretty cut and dried, right? The parents do not have autism, the kids do, and there are new mutations in genes that make sense for the autism phenotype. Yet, the authors did not stop there. They found that two of the four probands with putative causal de novo mutations also had inherited mutations that likely contributed to the phenotype. One possesses a de novo missense change in *SCN1A* in conjunction with a maternally inherited deletion at 15q11.2 that is known to increase risk of epilepsy and schizophrenia. Another has a truncating *FOXP1* mutation as well as an inherited, deleterious mutation in *CNTNAP2*, a gene that is negatively regulated by FOXP1. The truncated form of FOXP1 does not do this as well as it should, yielding increased expression of *CNTNAP2*. This lack of downregulation could exacerbate the effect of the *CNTNAP2* mutation, leading the authors to propose that multiple "hits" might cause some cases of sporadic autism.

O'Roak et al. Nature Genetics. Published online May 15, 2011. 10.1038/ng.835.

This Month in Our Sister Journals

High-Throughput Approach to *FMR1* Analysis for Use in Both Sexes

The broad size range for the fragile-X-syndrome-related CGG repeat in *FMR1* makes it a challenge to develop a single high-throughput diagnostic technique that will size repeats across this range and simultaneously assess their methylation status. Chen et al. recently developed a two-color PCR-based approach that improves the ability to do just that. In their approach, two aliquots of a genomic sample are prepared, and one is treated with a methylation-sensitive restriction enzyme. Each is then amplified using a fluorescent primer, and the two products are combined and analyzed through capillary electrophoresis using the two appropriate color channels. Methylated alleles are resistant to digestion, so the peak size for each allele can be compared in the digested and control reactions to measure the methylation fraction for each allele. Results based on this technique agree with those from Southern blot analysis for several tested cell lines and clinical samples, representing a range of allele sizes. Unlike some of the previously-proposed PCR-based techniques for *FMR1* analysis, this approach can be used in both males and females, and it yielded an unexpected finding in females with premutation-sized alleles; of 18 such samples, 15 exhibited size mosaicism for the premutation allele as well as a skewed methylation pattern at the repeat. Whether and how this ties in to premutation instability and/or to the penetrance of

FMR1 premutation-associated phenotypes remains to be discovered.

Chen et al. Genetics in Medicine. Published online March 25, 2011. 10.1097/GIM.0b013e31820a780f.

Fitness Test for Cancer Mutations

In order to better understand carcinogenesis, much effort has been directed recently at genomic analysis of cancers with comparisons to the constitutional genome of the affected individual. A key to the interpretation of these data is the determination of the "driver" mutations from the "passenger" mutations. In other words, which of the mutations actually contributed to the development of the cancer and which are simply along for the ride? Fischer et al. recently published an analytic method to make this distinction that uses germline fitness to assess mutations and target loci. Their analysis of cancer mutations in a set of kinase genes is used to assess their approach. They argue that this type of evolutionary-theory-based analysis will help to uncover novel cancer genes. They also find evidence that somatic mutation of putative tumor suppressor genes is generally more deleterious to fitness than is oncogene mutation, hinting that tumor suppressor mutations are more likely to drive cancer development.

Fischer et al. Genetics. Published online March 24, 2011. 10.1534/genetics.111.127480.